2683-Pos Board B453
R222Q Nav1.5 Mutation Associated with a New SCN5A-Related Cardiac Arrhythmia
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Using a candidate-gene approach, we detected a variant of SCN5A, encoding the cardiac Na^+ channel Nav1.5, by screening a family with cardiac arrhythmia resulting in frequent premature ventricular contractions (PVCs) and non-sustained ventricular tachycardia. Arrhythmia mechanism involved ectopic foci originating from the His-Purkinje system. The same mutation, leading to the R222Q substitution, was present in two additional unrelated families with the same associated cardiac phenotype. Exercise or hydroquinidine dramatically decreased the number of PVCs. To evaluate the functional incidence of this substitution, whole-cell patch-clamp experiments were performed on transfected COS-7 cells. The activation and inactivation curves were negatively shifted in the presence of the mutation ($V_{1/2act}$, WT: -30.6 ± 2.1 mV, n=9; heterozygous: -37.2 ± 1.6 mV, n=9; $p<0.05$; $V_{1/2inact}$, WT: -79.6 ± 0.7 mV, n=10; heterozygous: -82.2 ± 1 mV, n=9; $p<0.05$) whereas the current density was unchanged. The use of depolarizing-voltage ramp confirmed the increase and negative shift of the TTX-sensitive window current (potential of g_{max}, WT: -42.8 ± 0.5 mV, n=12; homozygous: -58.6 ± 1.1 mV, n=, $p<0.001$). WT and R222Q peak I_{Na} were similarly half-reduced by 30 µM quinidine ($p<0.001$ vs control) as well as the window current (WT: from -1.87 ± 0.42 to -0.74 ± 0.16 pA/pF in quinidine, n=7; homozygous: -2.54 ± 0.41 to -1.17 ± 0.12 pA/pF in quinidine, n=6; $p<0.001$ vs. control).
We carried out computer simulations in single-cell models of human Purkinje fibers and ventricle action potentials (AP). In heterozygous conditions, incomplete repolarization occurred in Purkinje cells only. We also built a multicellular model incorporating both cell models. In the heterozygous conditions, incomplete repolarization in the Purkinje fibers triggered premature APs propagating into the ventricle. This was normalized at higher pacing frequency or when quinidine was 'added'.
From all these studies, the premature ventricular contractions are explained by the appearance of electrical abnormalities rather in Purkinje fibers than in ventricular cardiomyocytes.

2684-Pos Board B454
Role of the Cytoplasmic N-Terminal Domain of the Cardiac Sodium Channel Alpha-Subunit
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Heterozygous loss-of-function mutations in the *SCN5A* gene encoding the cardiac Na^+ channel $Na_v1.5$ cause various hereditary arrhythmias including Brugada syndrome (BrS), a disease characterized by ST-segment elevation in the ECG right precordial leads, and an increased risk of sudden cardiac death. Here, we aimed to study the role of the $Na_v1.5$ evolutionarily conserved N-terminus of which the function remains unknown. We characterized two mutations within the cytoplasmic N-terminus of $Na_v1.5$, R104W and R121W identified in BrS patients and a construct where this region was deleted. In HEK cells, R104W, R121W and Nav1.5-ΔNter abolished I_{Na}. Immunostaining of Nav1.5 transfected in neonatal rat cardiomyocytes showed retention of R104W and R121W in cytoplasmic compartments, as opposed to the WT channels. Moreover, Western blot analysis revealed that mutants were mostly degraded and that the ubiquitin proteasome inhibitor, MG132, prevented this degradation. Coexpression of WT with either mutants, mimicking the heterozygous state of BrS patients, led to a marked I_{Na} density reduction (80%) and a 8 mV positive shift of the $V_{0.5}$ activation, compared to WT alone, demonstrating a dominant-negative effect. Interestingly, when $Na_v1.5$-ΔNter was coexpressed with WT, no dominant-negative effect was observed. We demonstrated that (i) the two N-terminal mutation abolish I_{Na} through retention of the mutant channels and their subsequent degradation by the proteasome, (ii) the two mutant channels exert a specific dominant-negative effect on WT channels by producing a default in WT channel trafficking to the membrane, (iii) these mutations have more drastic effects on I_{Na} than deletion of this region. Altogether, our results suggest that the N-terminal domain of Nav1.5 plays an important role in the traffic of Nav1.5 subunits to the plasma membrane, the regulation of their expression and their activation.

2685-Pos Board B455
The Role of Channel PKA/PKC Sites in Metabolic Regulation of the Cardiac Na^+ Channel
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Background: Intracellular NADH downregulates cardiac Na^+ current (I_{Na}) acutely to a magnitude seen in Brugada Syndrome by activating protein kinase C (PKC). The decrease in I_{Na} can be ameliorated by protein kinase A (PKA) activators. PKC and PKA down- and upregulates I_{Na}, respectively. Here, we studied the roles of known channel phosphorylation sites.
Methods: HEK293 cells transfected with human cardiac Na^+ channel wild type (WT), S1503A, S1503D, S525/528A, and RRR533-535AAA, were utilized for whole-cell patch clamp recording. Peak I_{Na} was measured at −30 mV with holding potential at −100 mV. All data were compared with WT.
Results: The peak I_{Na} of all mutants was similar to that of the WT channel. Compared to the WT $Na_v1.5$, the peak I_{Na} obtained with application of 100 µM NADH decreased to $41 \pm 5\%$ ($P<0.01$). Application of NADH failed to reduce I_{Na} of S1503A ($81 \pm 6\%$) but not of S1503D ($42 \pm 2\%$, $P<0.01$). PMA, a PKC activator, decreased I_{Na} of WT and S1503D ($47 \pm 5\%$ and $37 \pm 6\%$, respectively, $P<0.01$), but not that of S1503A ($87 \pm 11\%$, $P>0.05$). The NADH-induced decrease in I_{Na} could be ameliorated with the PKA activator, forskolin in S525/528A ($92 \pm 10\%$) but not RRR533-535AAA ($47 \pm 5\%$, $P<0.01$). The latter mutation also prevented NAD^+ mediated current recovery. **Conclusions**: The relatively retained current with the 1503 mutations alone and the lack of effect on I_{Na} by PKC activation of 1503A but not 1503D suggests that phosphorylation at this site is necessary but not sufficient to explain the NADH-dependent reduction in I_{Na}. The PKA-dependent rescue of current seems to be dependent on phosphorylation of the channel at the RRR533-535 sites.

2686-Pos Board B456
Functional Analysis of Stably Expressed Human Nav1.9
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The tetrodotoxin (TTX)-resistant voltage gated sodium channels, $Na_V1.8$ and $Na_V1.9$, are important for neuronal pain pathways. $Na_V1.9$ (a.k.a. NaN) is preferentially expressed in nociceptive neurons of dorsal root ganglia. The role of $Na_V1.9$ in inflammatory and neuropathic pain along with a restricted cellular localization makes it an attractive target for novel analgesics. Most of our knowledge about $Na_V1.9$ function comes from studies of rodent sensory neurons using internal solutions and recording protocols to differentiate $Na_V1.8$ and $Na_V1.9$ currents, as well as recent studies using neurons isolated from $Na_V1.8$-null mice. To further elucidate the functional properties of $Na_V1.9$, we developed a stable cell line expressing full length human $Na_V1.9$. ND7/23 cells were stably transfected using a novel transposon system in combination with human β1 and β2 sodium channel accessory subunits. Whole-cell currents were recorded from a holding potential of −120 mV and elicited with 20 ms pulses from −80 to +50 mV in the continuous presence of 200 nM TTX to block an endogenous TTX-sensitive sodium current. We recorded slow-activating, persistent sodium currents in $Na_V1.9$ expressing cells following 24 h incubation at 28°C (to boost cell surface expression). The whole-cell current peaked at −30 mV (15.2 ± 1.8 pA/pF, n=26) and exhibited a voltage-dependence of activation with V½ of −50.5 ± 1 mV and slope factor (k) of 7.2 ± 0.3 (n=26). Activation kinetics was much slower than that observed for neuronal, TTX-sensitive Na_V channels; time to peak current (at −30 mV) was 12.6 ± 0.7 ms, n=26. Preliminary single channel recording demonstrated frequent late re-openings and a single channel conductance of ~16 pS. These results demonstrate the biophysical properties of stably expressed human $Na_V1.9$ channel and provide a cell based platform for the analysis of potential $Na_V1.9$ therapeutic agents.

2687-Pos Board B457
Neuronal Sodium Channels Contribute Significantly to the Cardiac Repolarization during Ischemia
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Introduction: Infarcts are responsible for cardiac arrhythmias and transient ischemic episodes. It has been demonstrated that the neuronal sodium channels (nNa_vs) are overexpressed around an ischemic area. Ischemias induce a depolarization of the membrane potential and it's known that nNa_vs channels activate at more depolarized potentials than the cardiac sodium channels (Nav1.5). We hypothesised that this overexpression of nNavs channels may act as a safety factor for cardiac conduction during ischemia. However, the nNavs have a larger late component than Nav1.5 therefore prolonging the action potential duration (APD) and creating an arrythmogenic substrate (AS). The aim of this